

## **Supplementary Figure Legends**

### **Supplementary Figure 1. Schematic illustrating the generation of *Vav-iCre<sup>+/−</sup>;Ercc1<sup>−/−</sup>* mice.**

(a) The *Ercc1* WT allele is indicated with numerals over vertical lines denoting the 10 exons of *Ercc1*. LoxP sites in the floxed (*Ercc1<sup>fl/fl</sup>*) alleles are represented as black triangles. In the floxed allele, exons 7-10 were replaced by a cDNA cassette (grey rectangle) containing exons 7-10 and a *neo<sup>r</sup>* cassette. (b) Breeding scheme to generate *Vav-iCre<sup>+/−</sup>;Ercc1<sup>−/−</sup>* mice in an f1 genetic background that is 50:50 C57BL/6J:FVB.

**Supplementary Figure 2. *Vav-iCre<sup>+/−</sup>;Ercc1<sup>−/−</sup>* mice maintain normal weight and body composition.** (a) Body weight (BW) of three different age groups of mice by genotype. (b) Percent fat, (c) lean mass and (d) fluid measured by NMR (n=8-25 mice/group; Supplementary Table 3 has details). Values represent mean ± SD, one-way ANOVA with Tukey's test, not significant.

**Supplementary Figure 3. Quantitation of DNA adducts, cellularity and absolute lymphocyte numbers in the spleens of *Vav-iCre<sup>+/−</sup>;Ercc1<sup>−/−</sup>* mice.** (a) Levels of four cyclopurine adducts in splenic tissue from 8-10-month-old *Vav-iCre<sup>+/−</sup>;Ercc1<sup>−/−</sup>* mice and littermate controls (n=4-5 *Vav-iCre<sup>+/−</sup>;Ercc1<sup>−/−</sup>*; n=5 *Vav-iCre<sup>+/−</sup>*). Supplemental Table 3 has details) determined by LC/MS/MS/MS (see Methods). (b) Total splenocyte cell counts from 8-10-month-old *Vav-iCre<sup>+/−</sup>;Ercc1<sup>−/−</sup>* (n=14) mice and *Vav-iCre<sup>+/−</sup>* (n=12) mice. (c) The absolute number of CD4<sup>+</sup>, CD8<sup>+</sup> and B220<sup>+</sup>CD19<sup>+</sup> lymphocytes in spleens from the same animals (n=10/4 *Vav-iCre<sup>+/−</sup>;Ercc1<sup>−/−</sup>*; n=8/3 *Vav-iCre<sup>+/−</sup>* for CD4<sup>+</sup> or CD8<sup>+</sup>/ B220<sup>+</sup>CD19<sup>+</sup> measures, respectively). See Methods for experimental details. (d) Total splenocyte cell counts from young (7-month-old; n=10) and old (24-month-old; n=17) WT mice. (e) The absolute number of CD4<sup>+</sup>, CD8<sup>+</sup> and B220<sup>+</sup>CD19<sup>+</sup> lymphocytes in spleens from the same animals (n=8/3 young WT; n=17/7 old WT mice for CD4<sup>+</sup> or CD8<sup>+</sup>/ B220<sup>+</sup>CD19<sup>+</sup> measures, respectively). (f) Thymic weight at two ages (n=3 at 3-months-old; n=4 at 8-10-months-old per group). Weights were normalized to total body weight. Values represent mean ± SD, one-way ANOVA with Tukey's test. \*p<0.05, ^p<0.01, ^p<0.001.

**Supplementary Figure 4. Delayed-type hypersensitivity response by genotype and age.** (a) Schematic diagram of the delayed-type hypersensitivity (DTH) experiment to assess adaptive immune function in mice. Sensitization of the *Vav-iCre* mice began at 5-months-of-age. Old WT mice were ≥24-months-of-age. (b) Footpad swelling measurements at multiple timepoints following antigenic (KLH) challenge, broken out by animal genotype (n=7 *Vav-iCre<sup>+/−</sup>;Ercc1<sup>−/−</sup>*; n=6

*Vav-iCre<sup>+/</sup>*; n=5 old WT mice). (c) Footpad swelling by genotype in the same animals. (d) Quantitation of anti-KLH antibodies by ELISA 1-month post-antigenic challenge (n=3/7 *Vav-iCre<sup>+/</sup>*; *Ercc1<sup>-/-</sup>*; n= 3/6 *Vav-iCre<sup>+/</sup>*; n=3/3 *Ercc1<sup>-/-</sup>* for naïve (un-injected) and KLH-challenged (injected) mice, respectively). (e) DTH assay in 2-month-old mice *Vav-iCre<sup>+/</sup>*; *Ercc1<sup>-/-</sup>* (n=5) and *Vav-iCre<sup>+/</sup>* (n=8) littermate controls (blue). Values represent mean ± SD, two-way ANOVA with Tukey's test.  $\diamond p < 0.01$ ,  $\heartsuit p < 0.001$ ,  $\# p < 0.0001$ .

**Supplementary Figure 5. Sex-based differences in senescence marker and SASP expression in *Vav-iCre;Ercc1<sup>-/-</sup>* and aged WT mice.** (a) Senescence marker expression in the livers of 8-10-month-old *Vav-iCre<sup>+/</sup>*; *Ercc1<sup>-/-</sup>* (n=5♂ and 4-5♀; Supplemental Table 3 has details on n per gender and gene) and littermate control mice (n=3♂ and 3-4♀) as well as 4-month-old (n=3♂ and 3-4♀) and 2-year-old (n=6♂ and 4-5♀); WT mice. (b) Levels of circulating SASP factor proteins measured by multiplex ELISA in serum from *Vav-iCre<sup>+/</sup>*; *Ercc1<sup>-/-</sup>* (n=3♂ and 3-4♀) and *Vav-iCre<sup>+/</sup>* (n=3♂ and 3♀) mice. Values represent mean ± SD, two-way ANOVA with Tukey's test. \*p<0.05,  $\diamond p < 0.01$ ,  $\heartsuit p < 0.001$ ,  $\# p < 0.0001$ .

**Supplementary Figure 6. Quantitation of oxidative DNA adducts in peripheral tissues of *Vav-iCre* mice.** (a) Cyclopurine adducts were measured in the liver and kidneys of 8-11-month-old *Vav-iCre<sup>+/</sup>*; *Ercc1<sup>-/-</sup>* (n=5) and littermate control *Vav-iCre<sup>+/</sup>* (n=5 for liver and n=6 for kidney) by LC-MS/MS/MS (see Methods). (b) Markers of oxidative stress including 4-hydroxynonenal (HNE) protein adducts and the ratio of reduced to oxidized glutathione (GSH/GSSG) measured in the kidneys from 8-11-month-old *Vav-iCre<sup>+/</sup>*; *Ercc1<sup>-/-</sup>* and littermate control *Vav-iCre<sup>+/</sup>* mice (n=6 per group). HNE measure by ELISA. GSH/GSSG measured by chromogenic assay (see Methods) (c) 8-oxo-guanine DNA adducts (8-oxo-dG) measured in spleen, liver, and kidney of mice at various ages (n=5-6/5-6/5 *Vav-iCre<sup>+/</sup>*; *Ercc1<sup>-/-</sup>*; n=5-6/5-6/5 *Vav-iCre<sup>+/</sup>*; n=5/5/10 old WT mice for spleen/liver/kidney, respectively). Values represent mean ± SD, unpaired two-tailed Student's t-test (a,b) or two-way ANOVA with Tukey's test (c). \*p<0.05,  $\diamond p < 0.01$ ,  $\# p < 0.0001$ .

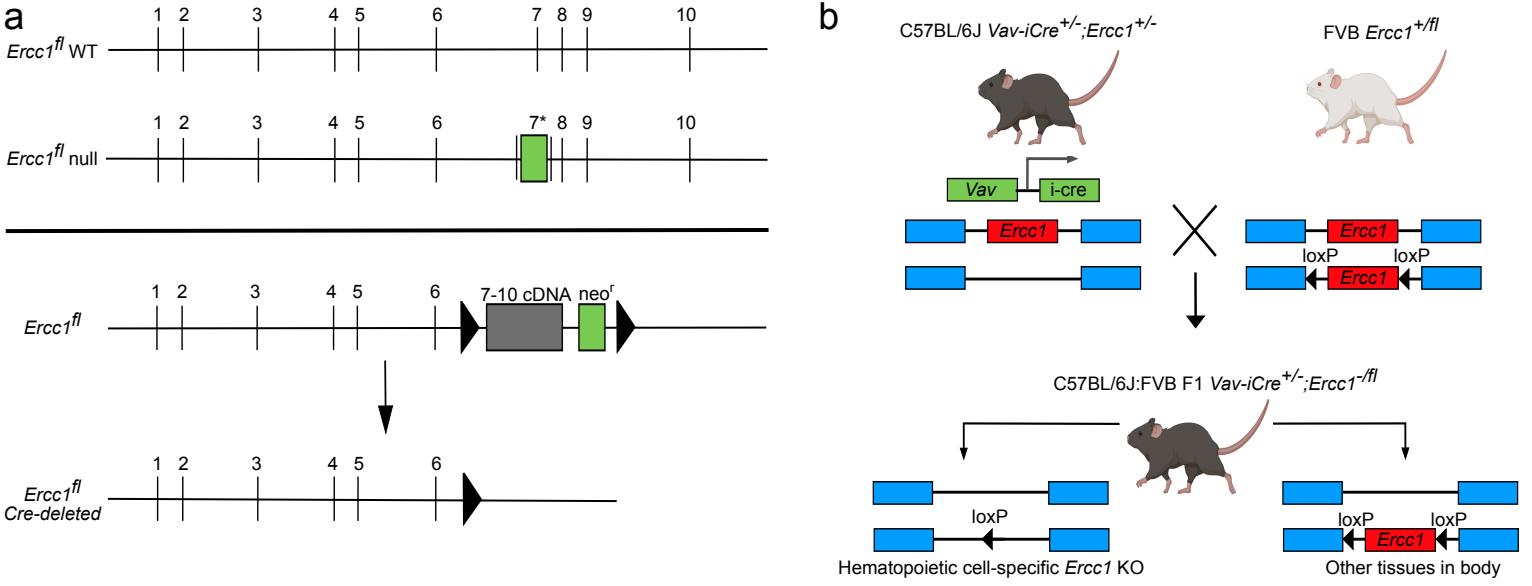
**Supplementary Figure 7. Uncropped immunoblots and gels.** (a) Immunoblots for Figure 1a and Extended Data Figure 1b. (b) Gels for Extended Data Figure 8b.

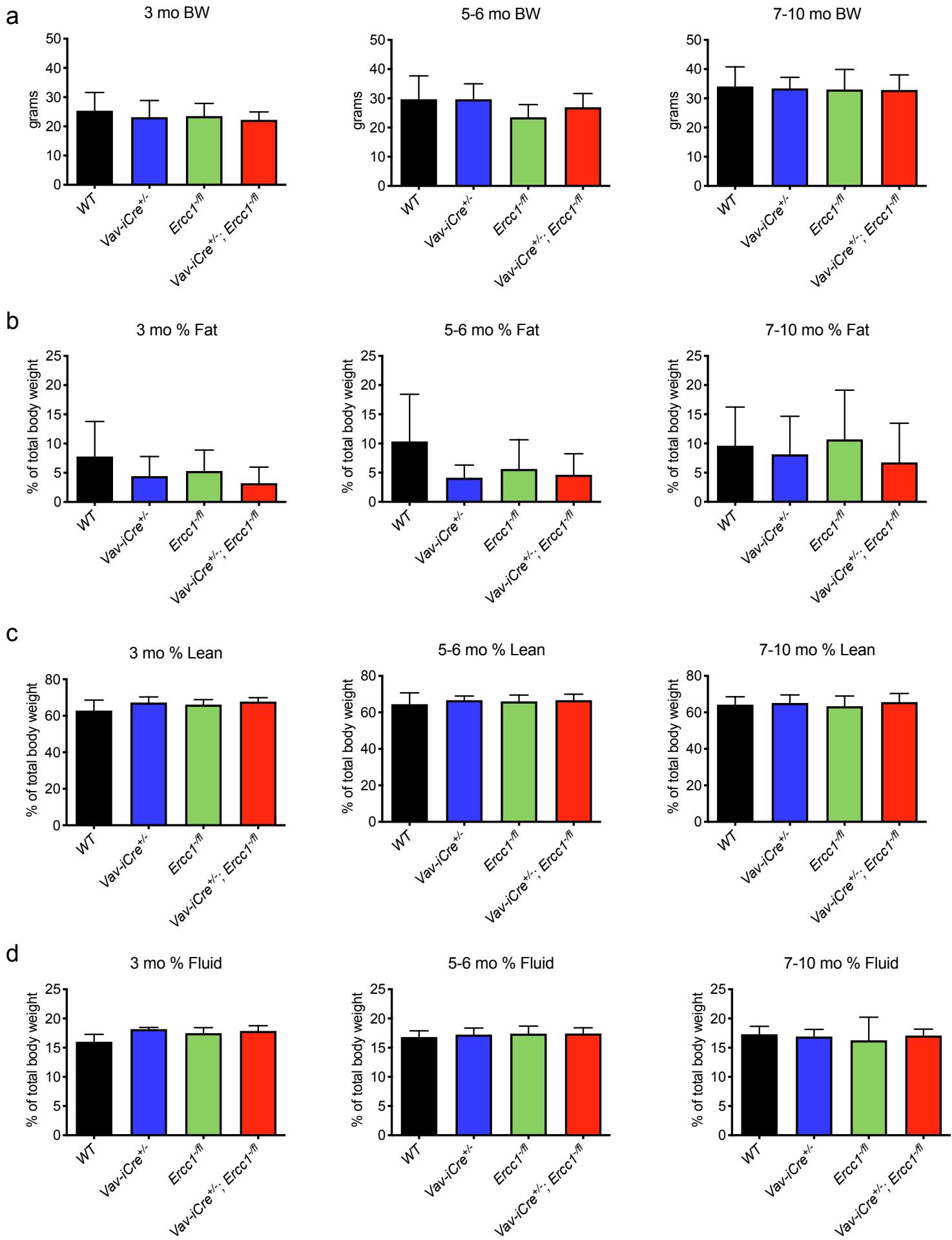
**Supplementary Table 1. Frequency of live births of mice by genotype.**

**Supplementary Table 2. Information about the antibodies used for CyTOF analysis.**

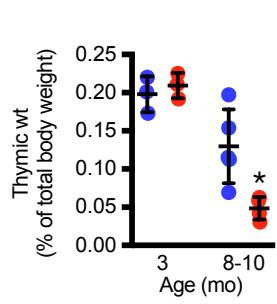
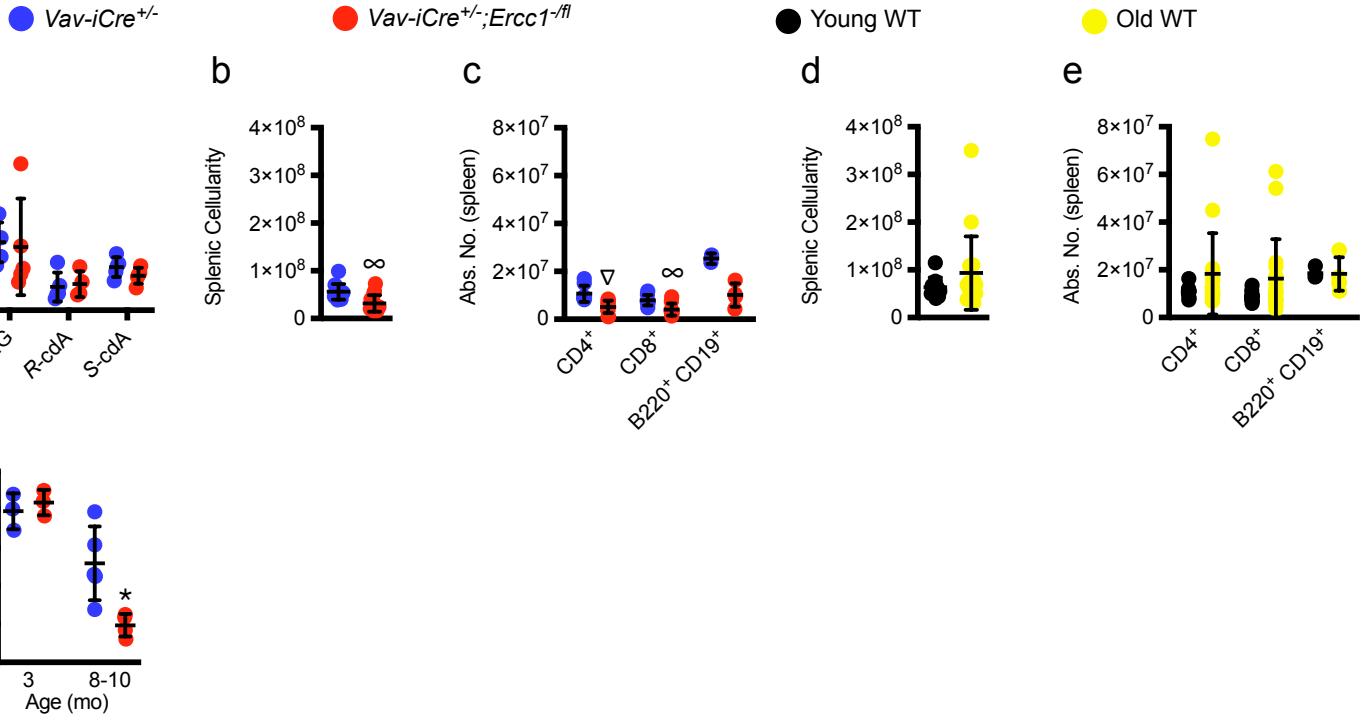
**Supplementary Table 3. Detailed information about the number of biologic replicates per experiment.**

**Supplementary Table 3. Experimental sample sizes.**

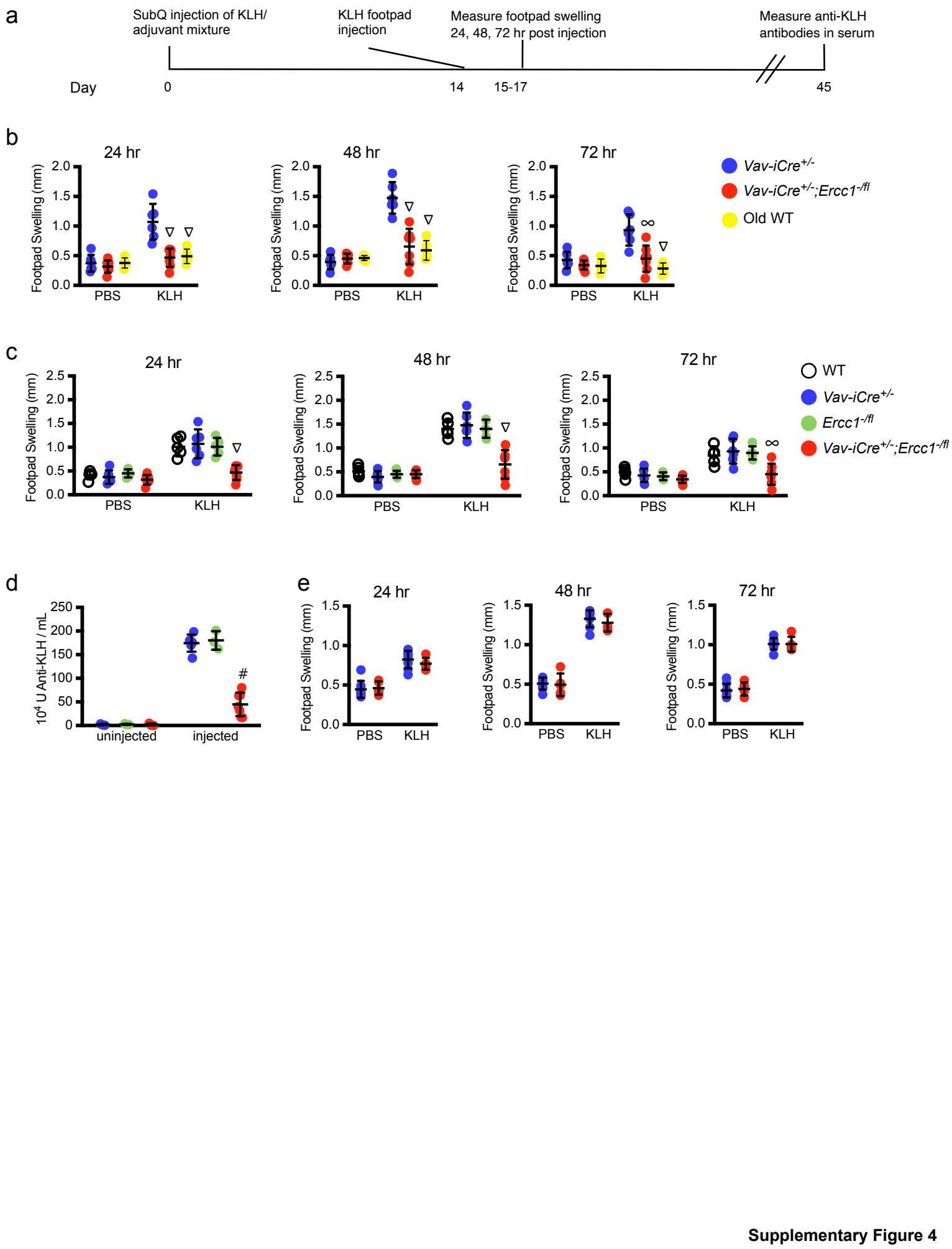


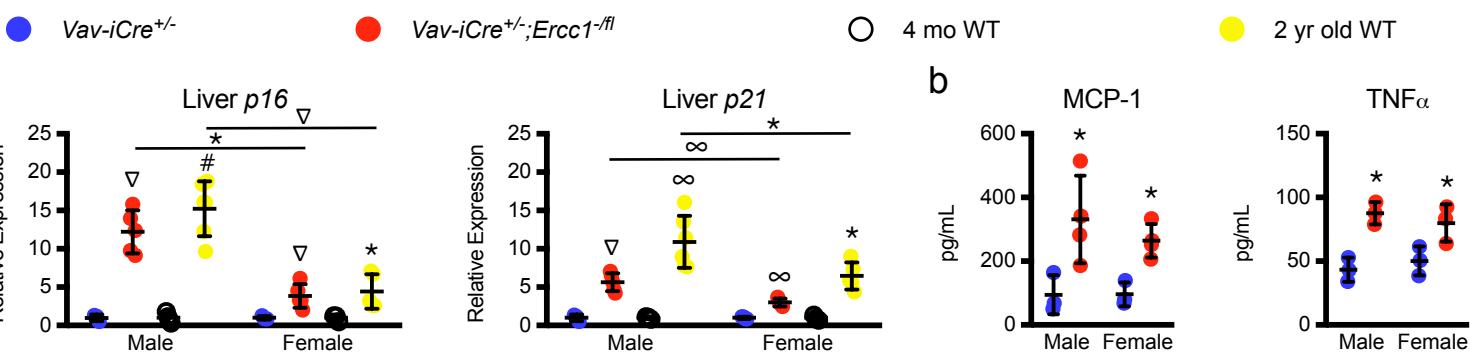


Supplementary Figure 2

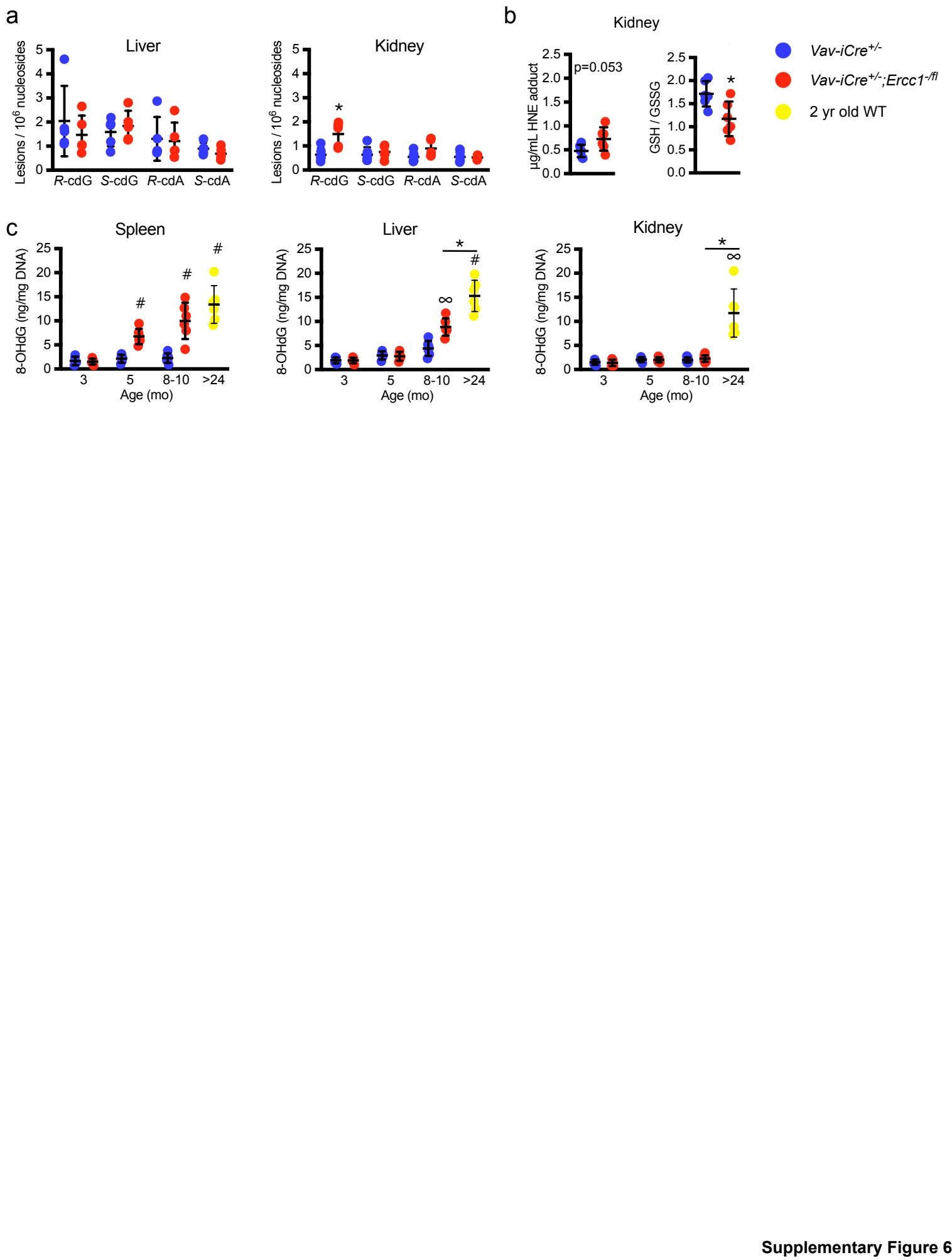


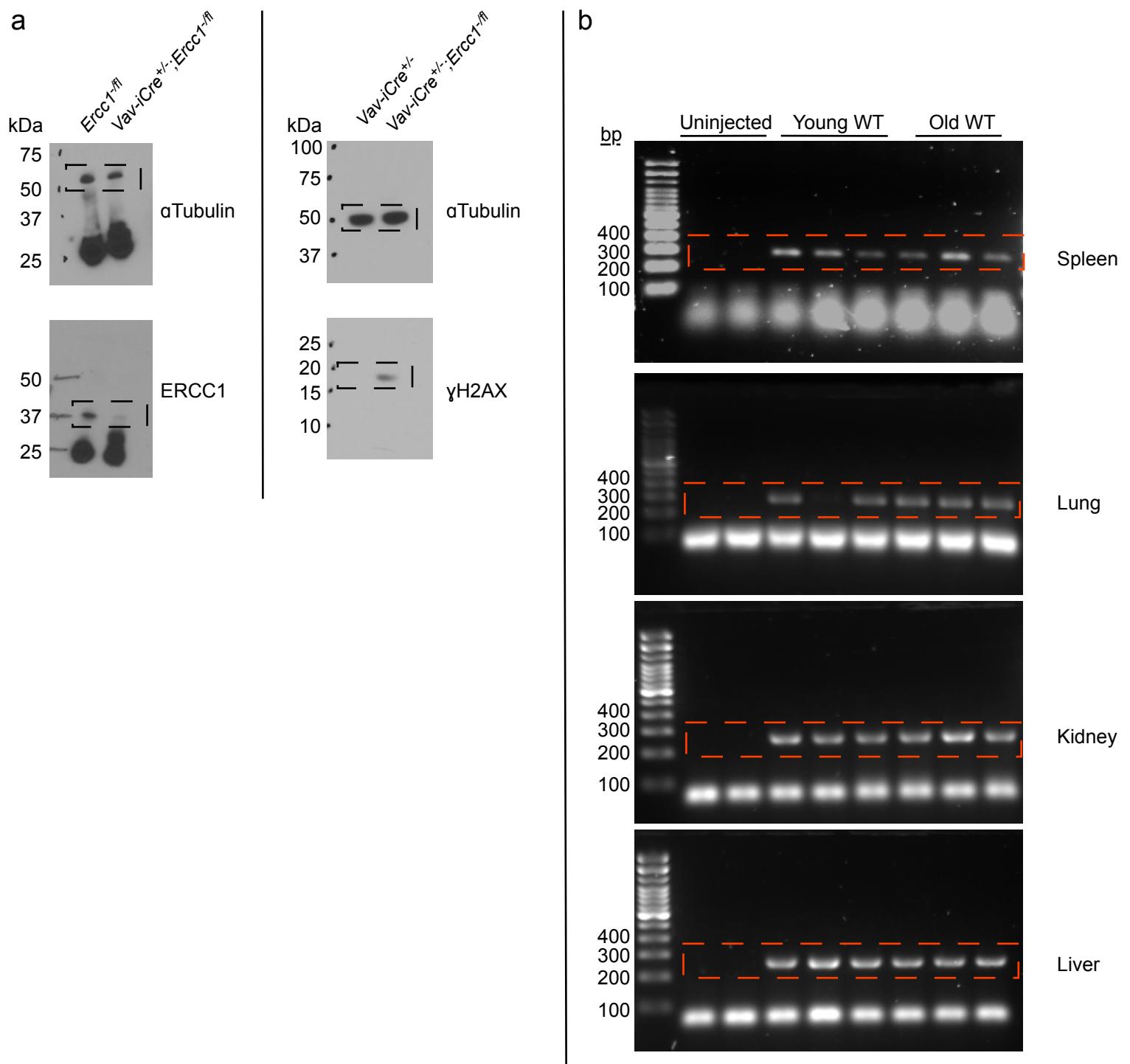
Supplementary Figure 3





Supplementary Figure 5





Supplementary Figure 7

**Supplementary Table 1: Mendelian frequency of births.**

Total Population				
Genetic Outcomes	Expected #	Observed #	Expected %	Observed %
Ercc1 -/c	63.5	64	12.5	12.6
Ercc1 +/-	63.5	57	12.5	11.22
Ercc1 +/c	63.5	72	12.5	14.17
Vav-iCre +/-	63.5	64	12.5	12.6
Vav-iCre +/-;Ercc1 -/c	63.5	57	12.5	11.22
Vav-iCre +/-;Ercc1 +/-	63.5	64	12.5	12.6
Vav-iCre +/-;Ercc1 +/c	63.5	65	12.5	12.8
WT	63.5	65	12.5	12.8
<b>TOTAL</b>	<b>508</b>	<b>508</b>	<b>100</b>	<b>100</b>

Chi-square test	
Chi-square	2.551
DF	7
P value (two-tailed)	0.9232

Female Population				
Genetic Outcomes	Expected #	Observed #	Expected %	Observed %
Ercc1 -/c	32.5	32	12.5	12.31
Ercc1 +/-	32.5	31	12.5	11.92
Ercc1 +/c	32.5	38	12.5	14.62
Vav-iCre +/-	32.5	31	12.5	11.92
Vav-iCre +/-;Ercc1 -/c	32.5	31	12.5	11.92
Vav-iCre +/-;Ercc1 +/-	32.5	39	12.5	15
Vav-iCre +/-;Ercc1 +/c	32.5	27	12.5	10.38
WT	32.5	31	12.5	11.92
<b>TOTAL</b>	<b>260</b>	<b>260</b>	<b>100</b>	<b>100</b>

Chi-square test	
Chi-square	3.446
DF	7
P value (two-tailed)	0.8409

Male Population				
Genetic Outcomes	Expected #	Observed #	Expected %	Observed %
Ercc1 -/c	31	32	12.5	12.9
Ercc1 +/-	31	26	12.5	10.48
Ercc1 +/c	31	34	12.5	13.71
Vav-iCre +/-	31	33	12.5	13.31
Vav-iCre +/-;Ercc1 -/c	31	26	12.5	10.48
Vav-iCre +/-;Ercc1 +/-	31	25	12.5	10.08
Vav-iCre +/-;Ercc1 +/c	31	38	12.5	15.32
WT	31	34	12.5	13.71
<b>TOTAL</b>	<b>248</b>	<b>248</b>	<b>100</b>	<b>100</b>

Chi-square test	
Chi-square	5.097
DF	7
P value (two-tailed)	0.6482

Male Vs. Female				
Genetic Outcomes	Expected #	Observed #	Expected %	Observed %
Ercc1 -/c	254	260	50	51.18
Ercc1 +/-	254	248	50	48.82
<b>TOTAL</b>	<b>508</b>	<b>508</b>	<b>100</b>	<b>100</b>

Binomial Test	
P (one-tailed)	0.3128
P (two-tailed)	0.6256
P value summary	ns

**Supplementary Table 2. CyTOF antibody information.**

No.	Label	Target	Clone	Localization	Company	Dilution Factor
1	089Y	CD45	30-F11	Surface	Fluidigm	200
2	141Pr	CD39	24DMS1	Surface	Biolegend	100
3	142Nd	Eomes	Dan11mag	Intracellular/Nuclear	Thermo Fisher	100
4	143Nd	TCRb	H57-597	Surface	Fluidigm	400
5	144Nd	Tcf1	812145	Intracellular/Nuclear	R&D Systems	200
6	145Nd	CD69	H1.2F3	Surface	Fluidigm	200
7	146Nd	Gata3	TWAJ	Intracellular/Nuclear	Thermo Fisher	100
8	147Sm	CENP-B	polyclonal	Intracellular/Nuclear	Abcam	100
9	148Nd	ROR gamma (t)	B2D	Intracellular/Nuclear	Abcam	100
10	149Sm	CD366 (Tim-3)	RMT3-23	Surface	Biolegend	100
11	150Nd	IRF4	IRF4.3E4	Intracellular/Nuclear	Biolegend	100
12	151Eu	CD25 (IL-2R)	3C7	Surface	Fluidigm	100
13	152Sm	CD3e	145-2C11	Surface	Fluidigm	400
14	153Eu	CD28	37.51	Surface	Biolegend	100
15	154Sm	BATF	D7C5	Intracellular/Nuclear	Fluidigm	200
16	155Gd	Tbet	4B10	Intracellular/Nuclear	Biolegend	100
17	156Gd	CD90.2/Thy-1.2	30-H12	Surface	Fluidigm	400
18	158Gd	FoxP3	FJK-16s	Intracellular/Nuclear	Fluidigm	100
19	159Tb	CD279 (PD-1)	RMP1-30	Surface	Fluidigm	100
20	160Gd	CD62L	MEL14	Surface	Fluidigm	100
21	161Dy	Ki-67	B56	Intracellular/Nuclear	Fluidigm	100
22	162Dy	p21	F5	Intracellular/Nuclear	SCBT	100
23	163Dy	CD4	RM4-5	Surface	Biolegend	200
24	164Dy	CD73	TY/11.8	Surface	Biolegend	100
25	165Ho	Thy1.1	OX-7	Surface	Biolegend	200
26	166Er	CD19	6D5	Surface	Fluidigm	200
27	167Er	CD38	90	Surface	Biolegend	100
28	168Er	CD8a	53-6.7	Surface	Fluidigm	400
29	169Tm	CD272 (BTLA)	6F7	Surface	Thermo Fisher	100
30	170Er	CD161 (NK1.1)	PK136	Surface	Fluidigm	200
31	171Yb	CD11b	M1/70	Surface	Biolegend	400
32	172Yb	Fas	SA367H8	Surface	Biolegend	100
33	173Yb	p16	polyclonal	Intracellular/Nuclear	Proteintech	100
34	174Yb	CD223 (LAG-3)	C9B7W	Surface	Biolegend	100
35	175Lu	iNos (NOS2)	CXNFT	Intracellular/Nuclear	Thermo Fisher	100
36	176Yb	CD44	IM7	Surface	Biolegend	400
37	209Bi	CD11c	N418	Surface	Fluidigm	100